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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/439,534	11/12/99	MOLLER	S 2312-103

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EXAMINER	
MEHTA,A	
ART UNIT	PAPER NUMBER
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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

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1- File Copy

Office Action Summary	Application No.	Applicant(s)	
	09/439,534	MOLLER ET AL.	
	Examiner	Art Unit	
	Ashwin Mehta	1638	

-- Th MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *39-47,59,60 and 72* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *39-46,59,60 and 72* is/are rejected.

7)☒ Claim(s) *47* is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15)☒ Notice of References Cited (PTO-892)

16)☒ Notice of Draftsperson's Patent Drawing Review (PTO-948)

17)☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *4 & 5* .

18)☐ Interview Summary (PTO-413) Paper No(s). _____ .

19)☐ Notice of Informal Patent Application (PTO-152)

20)☐ Other: .

DETAILED ACTION

Election/Restriction

1. Applicant's election with traverse of Group V, claims 39-47, 59, 60, and 72 in Paper No. 8 is acknowledged. The traversal is on the ground(s) that Applicants believe the claims of Groups I and II to be related enough to be subsets of each other, since the two sets of claims do not require all the elements of the other. Applicants make similar arguments for the relation between Groups III and IV, and V and VI. Applicants also urge that each of these pairs of Groups are related to each other, because they involve as part of the inventive concept the use of promoters and recombinases to cut a portion of a vector to effect a deletion of a gene. This is not found persuasive because the vectors of each group are different and the excision event catalyzed by the recombinase results in the removal of a different piece of DNA from each of the vectors. Not all of the excision events are excision of genes, and not all the events result in the same effect. For example, the invention of Group I results in the removal of a terminator region from a gene, resulting in the expression of a gene. The invention of Group III results in the removal of only one gene, which is neither the recombinase gene nor the gene that induces its expression. The invention of Group V results in the removal of multiple genes. The invention of each group then results in a different effect. Applicants argue that Groups V and Group VI are closely related, and that the only difference between claim 39 of Group V and claim 48 of Group VI is that claim 39 includes a requirement for a gene encoding a transcription factor. However, this transcription

factor forms part of the inducer that induces the recombinase gene of Group V. The absence of the transcription factor in Group VI makes it a different invention, since the recombinase gene can now be induced by any other mechanism. The invention of Group VI thereby has a different mode of operation, and would also require a different search.

The requirement is still deemed proper and is therefore made FINAL.

Claim Objections

2. Claim 60 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form.

Claim 60 limits the method of claim 59 by requiring the transgenic plant or plant cell to be selected prior to adding the inducer. However, selection of the transformant is an inherent step of all genetic transformation methods. One would not know if a transformant has been obtained without the selection step. The specification also admits, on page 8, lines 20-21, that the transformation of any plant species requires a selectable marker to identify individuals that have been successfully transformed. There would be no point in practicing the claimed method on plants that have not been transformed. Claim 60 therefore does not change the scope of the claim from which it depends.

3. Claim 47 is objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claim 46 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The recitations "strong promoter" and "weak promoter" in lines 1-3 of the claim renders it indefinite. The terms "strong" and "weak" are not defined in the specification when used to describe promoters. In the art the terms are used to describe the level of transcription that is driven by a promoter, and not necessarily an inducible promoter. For example, a constitutive double CaMV 35S promoter is a stronger promoter than the constitutive single CaMV 35S promoter. However, it is not clear that claim 46 uses the terms "strong" and "weak" in this fashion. Rather, the terms appear to describe a promoter that is induced to different levels by the same inducer. The specification, however, on page 9, line 26, to page 10, line 2, refers to such promoters as "high affinity" and "low affinity", rather than "strong" and "weak". It is suggested

that "strong" and "weak in claim 46 be replaced with --high affinity-- and --low affinity--, if this is what the claim is referring to.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claim 42 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claim is broadly drawn towards any vector comprising a gene of interest, a marker gene, a transcription factor gene, an inducible gene encoding a recombinase, and two recombination sites, wherein the recombination sites flank the transcription factor gene, marker gene and the inducible gene, wherein the transcription factor is the glucocorticoid receptor.

The specification teaches a combination of the Cre/lox site-specific recombination system with the GVG inducible system for the excision of specific DNA fragments from transgenic Arabidopsis plants (page 4, line 20 to page 5, line). The specification cites U. S. Patent Application No. 09/014,952, now U.S. Patent No. 6.063.985, Chua et al) in reference to the GVG system (page 4, line 22). The transcriptional induction system taught by Chua et al does not

use the entire glucocorticoid receptor protein but only its hormone-binding domain as a regulatory domain in a chimeric transcription factor (col. 3, lines 62-65). This transcription factor is GVG, and also contains the heterologous DNA-binding domain GAL4 and the transactivating domain VP16. GVG binds to promoters containing copies of the GAL4 upstream activating sequence (UAS) (col. 3, line 65-col. 4, line 5; col. 6, lines 27-38). Aoyama et al (Plant J., Vol. 11) teach that although an inducible system comprising the glucocorticoid receptor in transient plant cell expression system was demonstrated, a general and efficient system has not been constructed for transgenic plants (page 605). The instant specification does not teach the use of the entire glucocorticoid receptor as a transcription factor to be used with the claimed invention. In the absence of further guidance, it would therefore require undue experimentation by one skilled in the art to use the entire glucocorticoid receptor as the transcription factor for use with the claimed invention. It is suggested that "glucocorticoid receptor" in claim 42 be replaced with --GVG--. Given the breadth of the claim, unpredictability of the art and lack of guidance of the specification, undue experimentation would be required to make and use the claimed invention.

6. Claim 45 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claim is broadly drawn towards any vector comprising a gene of interest, a marker gene, a transcription factor gene, an inducible gene encoding a recombinase, and two

recombination sites, wherein the recombination sites flank the transcription factor gene, marker gene and the inducible gene, and wherein said recombination sites are mutant *lox* sites and have a lower affinity for CRE than does wild-type *lox*.

The specification teaches that one skilled in the art would use the claimed vector to excise the marker gene from a vector following transformation into a plant (page 8, line 19 to page 9, line 12). However, the specification does not enable one to use the claimed invention with mutant *lox* sites which are not readily recognized by the CRE recombinase. Albert et al teach that mutant *lox* sites with reduced binding affinity for the recombinase favors the stabilization of an integration event into a plant genome, over the reverse excision event (pages 650, 651-652). The mutant *lox* sites would therefore impede one from using the claimed invention to excise the genes flanked by the recombination sites. Mutant *lox* sites which favor an excision event over the integration event are not taught by the specification. Undue experimentation would be required by one skilled in the art to use mutant *lox* sites with reduced affinity to the CRE recombinase to excise the DNA flanking the recombination sites from the claimed vector. Given the breadth of the claims, unpredictability of the art, and lack of guidance of the specification, undue experimentation would be required by one skilled in the art to make and use the claimed invention.

7. Claim 46 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for induction when using the GVG system and under airtight conditions, does not reasonably provide enablement for other induction systems and under open air conditions. The

specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The claim is broadly drawn towards any vector comprising a gene of interest, a marker gene, a transcription factor gene, an inducible gene encoding a recombinase, and two recombination sites, wherein the recombination sites flank the transcription factor gene, marker gene and the inducible gene, and wherein said marker gene is under the control of a promoter that is induced by an inducer at low concentration and said recombinase gene is under the control of a promoter that is induced by said inducer at high concentration.

The specification teaches on page 9, line 45 to page 10, line 8, that "it is possible" to construct a site-specific recombination system in which both the marker gene and the recombinase gene are under the control of the same inducible promoter, but harbor high or low affinities towards the inducer. The marker gene has the high affinity, 6xUAS promoter, and the recombinase gene has the low affinity, 1xUAS promoter. These promoters are induced by the addition of the steroid hormone dexamethasone (DEX). DEX activates the transcription factor GVG, which binds to the UAS promoter (page 4, lines 28-31). The 6xUAS promoter would supposedly require a lower concentration of DEX for induction than the 1xUAS promoter. Therefore, following transformation, a low enough concentration of DEX will supposedly induce the marker gene without inducing the recombinase, and transgenic plants are selected. Addition of a higher concentration of DEX would then induce the recombinase and excise the region of the integrated vector harboring the marker gene. However, this system would be dependent on a

strict control of the concentration of the inducer, which may vary in a plant. Aoyama (in *Inducible Gene Expression in Plants*, 1999) teaches that it is uncertain to know how much DEX is taken up by plants in open air conditions. Aoyama teaches that glucocorticoid (DEX is an analog of glucocorticoid) is spread in the plant through the vascular tissues. However, under open air conditions glucocorticoid accumulates in leaves in higher concentrations as a result of transpirational water flow, and "it is very difficult to deliver glucocorticoid uniformly throughout a plant" (page 50). Aoyama suggest that one possible way of doing so is by growing enclosed plants on an agar medium containing DEX under airtight conditions (page 50). The specification does not teach any other such induction system. Neither does the specification teach the concentration of inducer that will not induce the lower affinity 1xUAS promoter while still inducing the 6xUAS promoter, especially given the tendency of inducers such as glucocorticoid and DEX to accumulate in plants. In the absence of further guidance, undue experimentation would be required by one skilled in the art to control the concentration of the inducer under all conditions. See Genentech, Inc. V. Novo Nordisk, A/S, 42 USPQ2d 1001, 1005 (Fed. Cir. 1997), which teaches that "the specification, not the knowledge of one skilled in the art" must supply the enabling aspects of the invention. Given the breadth of the claim encompassing the use of any inducer under any condition, unpredictability of the art and lack of guidance of the specification as discussed above, undue experimentation would be required by one skilled in the art to make and use the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

8. Claims 39-44, 59, 60, and 72 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ebinuma et al in view of Lyznik et al (Plant J., Vol. 8) and Aoyama et al (Plant J., Vol. 11).

The claims are broadly drawn towards any vector comprising a gene of interest, a marker gene, a transcription factor gene, an inducible gene encoding a recombinase, and two recombination sites, wherein the recombination sites flank the transcription factor gene, marker gene and the inducible gene; or a method for excising a marker gene from the genome of a transgenic plant or plant cell, comprising transfecting a plant or plant cell with said vector; or a plant or plant cell comprising said vector.

Ebinuma et al teach a method of producing a transgenic plant free of a marker gene, comprising introducing a vector into a plant cell, wherein said vector comprises a gene of interest, a marker gene, and a removable DNA element which can be removed by the action of a site-specific recombinase, wherein the marker gene and a recombinase gene are located within the removable DNA element (col. 4, line 10 to col. 5, line 27; col. 8, line 40 to col. 9, line 28; col. 21, line 15 to col. 24, line 17; claims).

Ebinuma et al do not teach an inducible recombinase gene.

Lyznik et al teach inducible expression of the FLP recombinase in maize cells, resulting in the excision of an NPT-II gene that had previously integrated into the genome; assert that the desired products of recombination cannot be stabilized unless the activity of a site-specific recombination system is regulated, wherein examples of such regulation includes using specific promoters; assert that applications of the FLP/FRT recombination system can be reduced to a single transformation step by using inducible promoters to conditionally activate the FLP recombinase (pages 178, 182).

Aoyama et al teach a glucocorticoid-mediated transcriptional induction (GVG) system that can be used in plants. Also taught are advantages of the system, including that glucocorticoid is non-toxic to plants; glucocorticoid can easily permeate plant cells, resulting in rapid gene induction; that the induction level can be regulated by using different concentrations of glucocorticoid, which allows one to examine dose-dependent effects of induced gene products (pages 606-610).

It would have been obvious and within the scope of one of ordinary skill in the art to modify the method marker gene excision of Ebinuma et al by placing the recombinase gene under the control of an inducible promoter, as suggested by Lyznik et al. One would have been motivated to regulate the recombinase gene with an inducible promoter given the assertions of Lyznik et al that the desired products of the recombination would not stabilize unless the recombinase is regulated, for example with an inducible promoter. The method may be further

modified by using the GVG system of Aoyama et al to control the induction of the recombinase gene. It would have been obvious to place the transcription factor gene within the segment that is removed by the recombinase, as it would no longer be required after the removal of the genes it regulates. One would be motivated to use the GVG system given its advantages taught by Aoyama et al.

8. Claim 47 is deemed free of the prior art, given the failure of the prior art to teach or fairly suggest motivation to include a chloroplast targeting peptide on the recombinase encoded by the inducible gene of the vector of claim 39.

9. No claim is allowed.

CLOSING REMARKS

Any inquiry concerning this communication should be directed to Examiner Ashwin Mehta, whose telephone number is (703) 306-4540. The Examiner can normally be reached Monday-Thursday and alternate Fridays, from 8:00 A.M. - 5:30 P.M. The fax phone number for the group is (703) 305-3014. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Paula Hutzell, can be reached at (703) 308-4310. Any inquiry of a

general nature or relating to the status of the application should be directed to the art unit's Patent Analyst, Gwendolyn Payne, whose telephone number is (703) 305-2475.

Ashwin D. Mehta

June 2, 2001

ASHWIN D. MEHTA, PH.D
PATENT EXAMINER